Gas Phase Studies of the Interactions of Fe^{2+} with Cysteine-Containing Peptides

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Gas-phase complexes of cysteine-containing peptides and Fe^{2+} were produced by fast atom bombardment and studied by tandem mass spectrometry. Specific and strong interactions of the iron and sulfur from the thiol group of the cysteine side chain are preserved in the gas phase and are the basis for highly specific fragmentation to give abundant $[a_n - 2H + Fe]^+$ ions, where n is position of the cysteine residue from the N-terminus of peptide. Metal/peptide complexes containing more than one Cys residue were also investigated; they display similar chemistry upon collisionally activated decompositions, indicating that the Fe^{2+} ion primarily binds at cysteine sites. (J Am Soc Mass Spectrom 1998, 9, 1285–1292) © 1998 American Society for Mass Spectrometry

The function of iron-containing proteins is determined by the properties of the metal center and its surrounding environment [1–5]. The structural, redox, and ligand-exchange properties of the iron center can be strongly altered by the ligand field of the coordination sphere [4]. High-spin octahedral ferrous complexes are exchange labile, whereas the corresponding axially ligated porphyrin complexes are spin paired (diamagnetic) and inert to ligand exchange. Large, bulky ligands, such as those provided by metalloproteins and enzyme sites, bind in a tetrahedral environment in which both Fe^{2+} and Fe^{3+} ions form high-spin complexes. The most preferred ligands for tetrahedral coordination are thiol groups such as those of a cysteine side chain [6, 7]. Knowledge of the interactions of Fe^{2+} ions with sulfur ligands and of the structures of the resulting complexes is essential for understanding electronic structures and oxidation–reduction properties of Fe^{2+}-containing proteins.

Often metal–ion binding sites are in the interior of proteins and are composed of a number of side-chain ligands that surround and interact simultaneously with the metal ion. The specificity of metal–ion binding to protein groups is determined by the intrinsic affinity of the peptide ligands and by solvent effects. The study of gas-phase metal-ion/peptide interactions, therefore, is appropriate for dissecting solution properties into intrinsic and solvation effects.

We are investigating the gas-phase interactions of metal ions with relatively low molecular weight peptides that closely mimic biological ligands in terms of composition, ligand types, and structure. We are interested in the study of metal/peptide complexes in the gas phase because they provide not only an understanding of intrinsic effects but also a foundation for new approaches to structure determination of peptides [8–22]. Most recent efforts in this emerging mass spectrometric area have been directed at the interactions of small peptides with alkali [8–13], alkaline-earth [14–18], and transition metals [19–28].

We reported recently in a communication [29] that specific and strong interactions of Fe^{2+} ions with the thiol groups of the Cys side chain are preserved in the gas phase and lead to the formation of stable $[M - H + Fe]^+$ complexes. CAD of these complexes resulted in the formation of $[a_n - 2H + Fe]^+$ fragment ions, where n is position of Cys residue in the peptide chain. This interesting finding motivated us to conduct further investigations of the interactions of Fe^{2+} ions with cysteine-containing peptides. In this article, we show that the complexes formed from a series of peptides ranging from penta to decamers containing one or more Cys at various positions decompose in highly specific way and reveal the location of the Fe^{2+} binding site.

Experimental

Reagents

The peptides used for this work were from the Immunology Program, Department of Pathology, Washington University School of Medicine, and were prepared by automatic stepwise solid-phase peptide synthesis from Fmoc-protected amino acids. The crude peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) by using a C-18 column, and collected fractions were lyophilized. The identity of the peptides was confirmed by fast-atom bombardment (FAB) mass spectrometry and tandem mass spectrometry of the $[M + H]^+$ ions. A 1:1 mixture of glycerol and

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thioglycerol saturated with $Fe(NO_3)_2$ was used to produce the metal/peptide complexes. The reagents for the matrix were obtained from Sigma (St. Louis, MO).

Instrumentation

Mass spectrometric experiments were performed on VG ZAB-T (Manchester, UK), four-sector [30] and Kratos MS-50, triple-sector mass spectrometers. The VG ZAB-T tandem mass spectrometer consisted of two high-mass, double-focusing mass spectrometers of overall BEBE design. FAB mass spectra were acquired after adjusting the mass resolving power of MS1 to approximately 1200 (10% valley definition). The instrument was operated at an accelerating voltage of 8 kV. FAB was by a Cs^+ gun that provided a 30-keV Cs^+ beam (the overall energy for desorption was 22 keV). The second stage, which is a reverse-geometry, Mattauch–Herzog type instrument with a planar electrostatic analyzer, was used to obtain product-ion mass spectra of precursor ions isolated by MS1. Ions were activated by collisions with helium gas at pressures sufficient to give 50% main-beam suppression; the collision cell was floated at 4 kV. The fragment ions formed in the third-field-free region were detected by a single-point detector. Data acquisition was carried out with a VG OPUS V 3.1X data system, which was interfaced to the mass spectrometer by means of a VG SIOS I unit.

The Kratos MS-50 tandem mass spectrometer, which was a forward geometry EBE instrument, was equipped with a Kratos FAB ion source and an Ion Tech saddle-field atom gun (Ion Tech, Middlesex, England), which produced a 6-keV Ar atom beam for FAB desorption. Collision cells were located in both field-free regions: between ESA-1 and the magnet and between the magnet and ESA-2. For acquiring product-ion mass spectra, MS-1 (ESA-1 and the magnet) were used to select the precursor ion, and a mass-analyzed ion kinetic energy scan (MIKES) was conducted by scanning the field of ESA-2. When MS/MS/MS experiments were performed, the ions (m_1) produced in the source were activated in the first collision cell, and a specific fragment ion (m_2) was selected by adjusting the ESA-1 to E/E_0 and the magnet to B/B_0, where E is $(m_2/m_1) \times E_0$, B is $(m_2/m_1) \times B_0$, and E_0 and B_0 are electric and magnetic-field strengths, respectively, of the precursor ions (m_1), allowing the ion of interest to be transmitted. The CAD spectra then were recorded by scanning the ESA-2. Helium was used as collision gas and was introduced to a pressure sufficient to reduce the precursor-ion beam by 50%.

Procedures

For acquiring both FAB full-scan and tandem mass spectra, 1 μL of the matrix was mixed with 1 μL of a 1-μg/μL peptide solution. The probe was admitted to the ion source and bombarded with the Cs^+ or Ar atom beam, depending on the instrument, to cause desorption. Usually, 10–20 scans were acquired and signal averaged to obtain a mass spectrum of the product ions.

The deuterium-labeled peptides were prepared on the FAB probe tip by using D_2O (Sigma) as a solvent, which was added to the mixture of the peptide and iron-containing matrix. The volatile H_2O, HDO, and D_2O were pumped away in the vacuum interlock of the FAB source. To obtain nearly complete isotopic exchange, the procedure was repeated three times.

Four Fe^{2+}/peptide complexes of GCCAL, AVAGCL, GCACLV, and GCACVCL were also formed by electrospray ionization and submitted to low-energy collisional activation (LECA) on ion-trap instrument (Finnigan LCQ, San Jose, CA). The electrospray conditions for the production of $[M - H + Fe]^+$ were the same as for the production of $[M + H]^+$, except the carrier solution, which was aqueous 0.2-mM $FeCl_2$ instead of 50:50 H_2O:MeOH. The samples were introduced at a flow rate of 10 μL/min. The spray needle was held at 4.2 kV, and a 70 lb/in.^{2} coaxial flow of nitrogen was used to stabilize the spray. A heated (200 °C) stainless-steel capillary served as the counterelectrode and was held at 13.5 V. In all experiments, helium was introduced to a pressure of 1 mtorr (measured by a remote ion gauge) for improving the trapping efficiency of the ion trap. The background helium gas also served as the collision gas. Collisional activation of the ions, which were excited by a tickle voltage (20 V) applied to the end caps, proceeded via multiple, low-energy collisions with helium. The isolation width and time for the collisional activation experiments were 1 u and 300 ms, respectively.

Results and Discussion

We investigated the interaction of cysteine-containing peptides with Fe^{2+} by desorbing with FAB the peptide/metal complexes into the gas phase as $[M - H + Fe]^+$ species and then submitting the desorbed species to collisional activation. For transition metal/peptide complexes, the amino-acid side chains usually provide the ligating atoms [25, 26, 29, 31, 32], although deprotonated nitrogens and carbonyl oxygens probably also serve as ligands [17–20]. Sulfur is the preferred biological ligand for Fe^{2+}.

Fragmentation of Iron/Peptide Complexes Containing Hexa to Decapeptides

We showed previously [29] that Cys-containing pentapeptides/iron complexes decompose upon CAD to give abundant a_n^* ions, where n is the position of the Cys residue with respect to the N-terminus of the peptide backbone. The nomenclature we now use is a simple adaption to the standard one for peptides. We omit the metal from the labels and designate product ions as if they were formed from protonated precursors;

Table 1. Peptide sequence and major fragment ions for Fe^{2+}-binding studies

Sequence		Major fragment ions		
Peptides with **one** Cys residue				
AVAGCL	$-H_2O$	$(a_5 - 2H + Fe)^+$		
VGACAL	$-H_2O$	$(a_4 - 2H + Fe)^+$		
AGVCAGV	$-H_2O$	$(a_4 - 2H + Fe)^+$		
AGVACGV	$-H_2O$	$(a_5 - 2H + Fe)^+$		
AGVAGCV	$-H_2O$	$(a_6 - 2H + Fe)^+$		
AGVACGVL	$-H_2O$	$(a_5 - 2H + Fe)^+$		
AGVAGCVL	$-H_2O$	$(a_6 - 2H + Fe)^+$		
AGVAGVCL	$-H_2O$	$(a_7 - 2H + Fe)^+$		
AGVAGVAGCL	$-H_2O$	$(a_9 - 2H + Fe)^+$		
Peptides with **two** Cys residues				
GCCAL	$-H_2O$	$(a_3 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_3 - 2H + Fe - H_2O)^+$	$-H_2S$
GCCAL-NH_2	$-H_2O$	$(a_3 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_3 - 2H + Fe - H_2O)^+$	$-H_2S$
AGCCAL	$-H_2O$	$(a_4 - 2H + Fe)^+$ $(a_3 - 2H + Fe)^+$	$(a_4 - 2H + Fe - H_2O)^+$	$-H_2S$
GAVCCL	$-H_2O$	$(a_5 - 2H + Fe)^+$ $(a_4 - 2H + Fe)^+$	$(a_5 - 2H + Fe - H_2O)^+$	$-H_2S$
GCACLV	$-H_2O$	$(a_4 - 2H + Fe)^+$	$(a_2 - 2H + Fe)^+$	$-H_2S$
ACGVCL	$-H_2O$	$(a_5 - 2H + Fe)^+$	$(a_2 - 2H + Fe)^+$	$-H_2S$
ACGVACL	$-H_2O$	$(a_6 - 2H + Fe)^+$	$(a_2 - 2H + Fe)^+$	$-H_2S$
Peptides with **three** Cys residues				
GCACVC	$-H_2O$	$(a_4 - 2H + Fe)^+$	$(a_2 - 2H + Fe)^+$	$-H_2S$
GCACVCL	$-H_2O$	$(a_6 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_4 - 2H + Fe)^+$	$-H_2S$
GCCACV	$-H_2O$	$(a_5 - 2H + Fe)^+$ $(a_3 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_3 - 2H + Fe - H_2O)^+$	$-H_2S$
GCCCAV	$-H_2O$	$(a_4 - 2H + Fe)^+$ $(a_3 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_4 - 2H + Fe - H_2O)^+$ $(a_3 - 2H + Fe - H_2O)^+$	$-H_2S$
Peptides with **four** Cys residues				
GCCCCA	$-H_2O$	$(a_5 - 2H + Fe)^+$ $(a_4 - 2H + Fe)^+$ $(a_3 - 2H + Fe)^+$ $(a_2 - 2H + Fe)^+$	$(a_4 - 2H + Fe - H_2O)^+$ $(a_3 - 2H + Fe - H_2O)^+$	$-H_2H$
Peptides with **no** Cys residues[a]				
AAVGAVL	$-H_2O$	x_6, a_6	y_5, w_5, c_5, b_5, a_5	c_4, b_4, a_4

[a]All ions contain Fe^{2+}.

for example, ions that contain Fe^{2+} are designated by an * such that, an a_n^* ion is $[a_n - 2H + Fe]^+$.

Here we ask the question whether gas-phase complexes of Fe^{2+} ions bound to the hexa, hepta, octa, and decapeptides containing one Cys residue in the peptide chain fragment in a manner that is consistent with that of the pentapeptides [29]. If they do, then the iron/peptide interaction may provide a general method to indicate the location of a cysteine residue in a peptide. Tandem mass spectrometry of FAB-produced ions reveals that strong and specific interactions of Fe^{2+} ions with the Cys side chain are preserved in the gas phase for 20 additional peptides that contain six or more amino acids (Table 1).

Just as Cys-containing pentapeptide/iron complexes decompose upon CAD to give abundant a_n^* ions, so do hexamers (e.g., VGACAL and AVAGCL) give a highly specific fragmentation at the periphery of the Cys residue to produce a_4^* and a_5^* ions of m/z 357 and 428, respectively (Figure 1). The hexapeptides probably bind Fe^{2+} in the same tetrahedral fashion; that is, sulfur is the primary binding ligand and nitrogens are the secondary ligands. The simplicity of the CAD spectra of peptide/iron complexes suggests that the number of fragmentation channels is significantly fewer than those of protonated peptides (compare Figure 1A, B with Figure 1C). Because protonation of peptides is not very specific, cleavages of the $[M + H]^+$ ion of VGACAL are typical and take place almost at every peptide bond to give principally b and a and, to a lesser extent, y and v sequence ions. The fragmentation channels for the decomposition of $[M - H + Fe]^+$ complexes, on the other hand, are determined by the site of metal attachment and are very specific. By providing a primary binding site (an anchor) for the metal ion and secondary chelation with amide nitrogens, the peptides strongly bind Fe^{2+}, and all fragment ions retain the metal. Besides sulfur ligands, other bonding atoms such as nitrogens

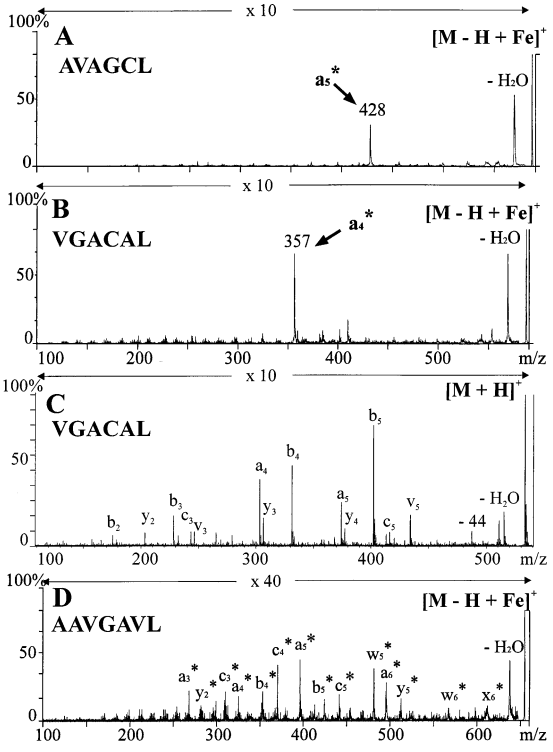


Figure 1. The CAD mass spectra of $[M - H + Fe]^+$ complexes of peptides: (**A**) AVAGCL, (**B**) VGACAL (m/z 587), and (**D**) AAVGAVL (m/z 654) and CAD mass spectrum of $[M + H]^+$ ion of (**C**) VGACAL (m/z 533).

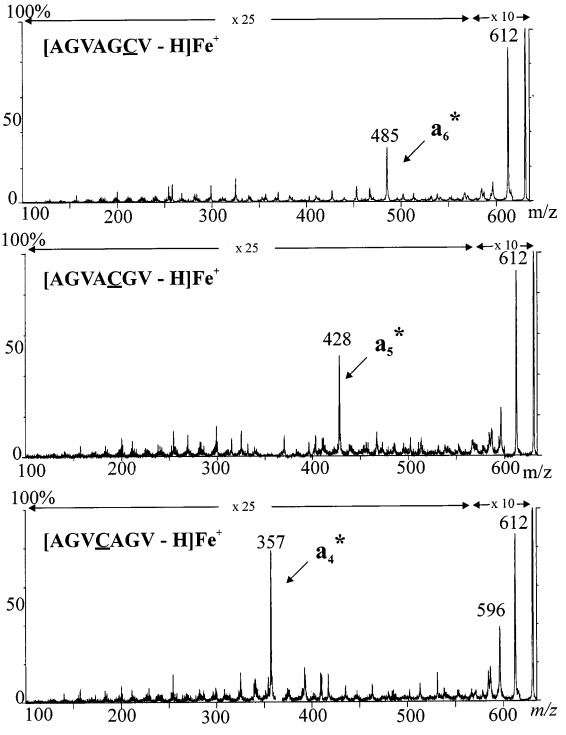


Figure 2. The CAD mass spectra of $[M - H + Fe]^+$ complexes of heptapeptides: (**A**) AGVAGCV, (**B**) AGVACGV, and (**C**) AGVCAGV (m/z 630).

are found in the iron–sulfur centers. An example is a Rieske center [23], where two imidazole nitrogens, in addition to the sulfur, are bound to the Fe atom. The proposed structure for these centers is tetrahedral, as was suggested for the sulfur–iron centers [23].

To verify that S serves as the anchor, a peptide containing only amino acids with aliphatic side chains (AAVGAVL) was studied. The full-scan FAB mass spectrum of AAVGAVL desorbed from an Fe^{2+}-containing matrix is dominated by the $[M + H]^+$ ion of the peptide, and the relative abundance of the $[M - H + Fe]^+$ ion is less than 10%, whereas the iron/peptide complexes and the $[M + H]^+$ ions are of comparable abundances for Cys-containing peptides [29]. If Fe^{2+} has no preferential binding to the peptide, it will not bind as well as the proton, and its weak binding will be nonspecific, allowing many equally favorable Fe^{2+}/peptide complexes to exist, each of which has its own fragmentation pathways. This nonspecific binding of Fe^{2+} to the peptide results in more complicated CAD spectra than those of Fe^{2+}-bound peptides containing one or more cysteines (Figure 1D). Clearly the SH of Cys is a principal binding site or anchor for gas-phase Fe^{2+}. The evidence does not rule out, however, competitive binding of Fe^{2+} to other functional groups (e.g., OH, NH_2).

Complexes of Fe^{2+} with hepta, octa and decapeptides decompose in a fashion that is similar to that of penta and hexapeptides. The expected a_n^* ions are formed, but their abundances relative to that of the precursor ion decreases as the Cys is moved toward C-terminus (e.g., $a_4^* > a_5^* > a_6^*$). For heptapeptides containing a Cys residue at position 6 from the N-terminus, the abundance of the a_6^* ion is two times smaller than that of a_4^* from a peptide with Cys at position 4 (Figure 2). Formation of a* ions may be less favorable for peptides with the Cys residue remote from the N-terminus because the simultaneous interaction of Fe^{2+} with the terminal NH_2 and the thiol group of cysteine requires folding of the peptide backbone around the metal center to give a macrocycle. This is entropically less favorable than forming a complex when the thiol group and the N-terminal NH_2 are more proximate.

Bonding of Fe^{2+} to Peptides Containing Two or More SH Groups

Upon CA, complexes of Fe^{2+} with peptides of the composition GCACLV and GCACVC decompose to give abundant a_2^* ions of m/z 187 and a_4^* ions of m/z 360. These fragmentations of hexapeptides containing two Cys that are separated by one residue are similar to those reported for the pentapeptide GCACL [29]. The modest extension of the chain from penta to hexapeptides does not cause any change in fragmentation because the various ligand sites that bind to the metal remain the same. Furthermore, incorporation of a third

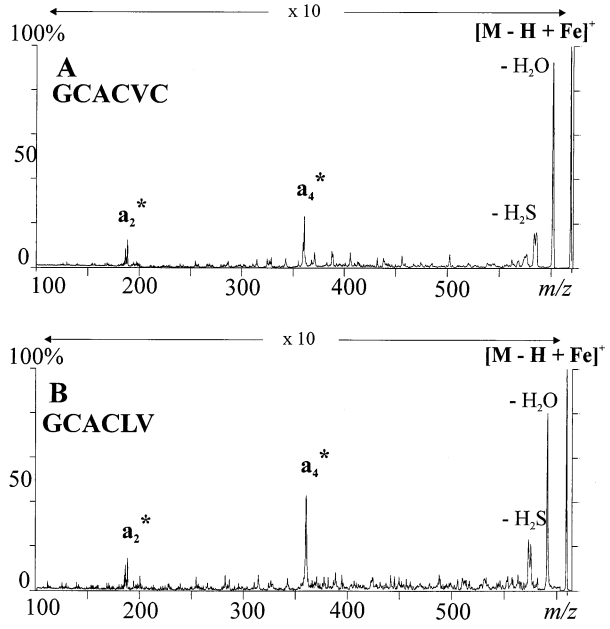


Figure 3. The CAD mass spectra of $[M - H + Fe]^+$ complexes of hexapeptides: (**A**) GCACVC (m/z 609) and (**B**) GCACLV (m/z 619).

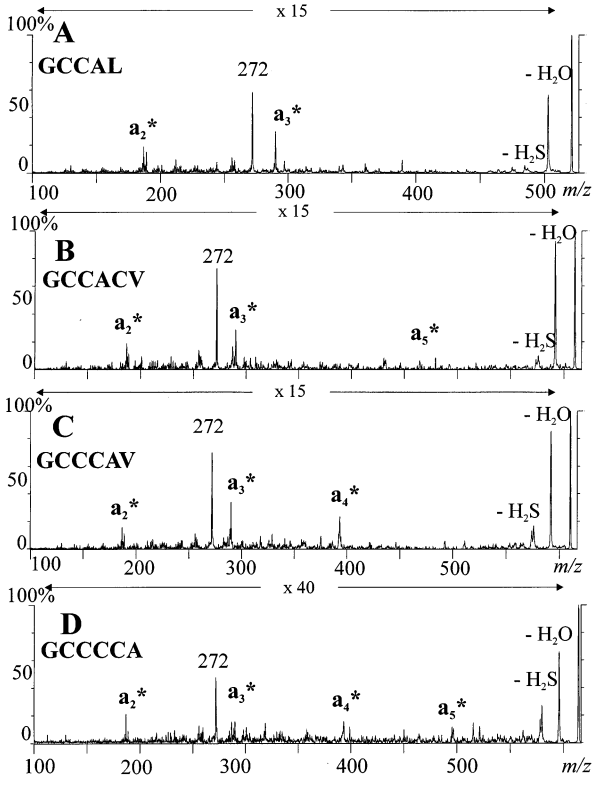


Figure 4. The CAD mass spectra of $[M - H + Fe]^+$ complexes of peptides: (**A**) GCCAL (m/z 520), (**B**) GCCACV, (**C**) GCCCAV (m/z 609), and (**D**) GCCCCA (m/z 613).

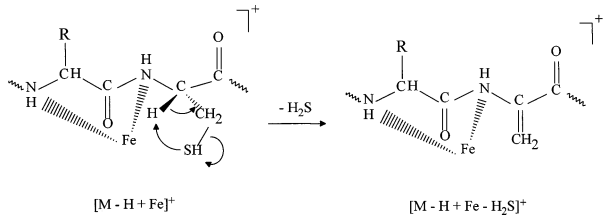
cysteine residue into the peptide at the C-terminus does not affect the fragmentation scheme (Figure 3). This is more evidence that the N-terminal amino group, in addition to the SH group, is involved in bonding to Fe^{2+}.

Peptides that contain cysteine residues remote to each other (e.g., ACGVCL and ACGVACL) are less prone to fragment to an a_n^* ion upon binding to the iron. Facile water loss from the $[M - H + Fe]^+$ ion is the major fragmentation channel. Other low-abundance ions are a_2^* of m/z 201 for both iron/peptide complexes, a_5^* of m/z 460 for ACGVCL, and a_6^* of m/z 531 for ACGVACL. The relatively low abundance of a* ions for these complexes, compared to those with one Cys residue, suggest that both cysteine functional groups are involved in the binding to the iron. Even though the peptide backbone may be broken by "Cys-directed" cleavage to give an a* ion, simultaneous interaction of iron with the second cysteine holds the complex together. Multiple Cys-directed cleavages are required to fragment the iron/peptide complex. Because multiple cleavages are less probable and may require additional activating collisions than can occur in a tandem four-sector instrument, formation of these ions is not favored.

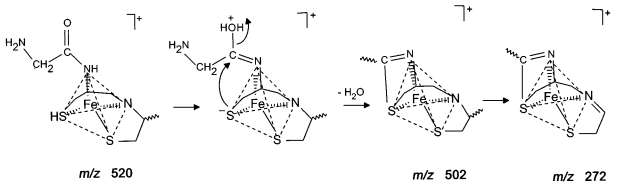
When the Cys residues adjoin each other, the fragmentation of iron/peptide complexes is similar to that of peptides with two Cys residues that are separated by one residue. The expected a_2^* ions of m/z 187 and a_3^* ions of m/z 290 are produced for GCCAL, GCCACV, GCCCAV, and GCCCCA peptides. An additional a_5^* fragment ion of m/z 464 arises for the peptide GCCACV, an a_4^* ion of m/z 393 for the GCCCAV, and a_4^* of m/z 393 and a_5^* of m/z 496 for the iron-bound complex of GCCCCA (Figure 4). Besides the expected iron-containing a ions, an ion $[a_3^* - H_2O]$ of m/z 272 is produced upon CA of these peptides. These observations verify that Fe^{2+}/peptide interactions can be used to indicate the locations of most cysteine residues in peptides with multiple Cys residues, although the signal-to-noise ratios for some a_n^* ion signals are low.

Losses of Small Molecules from the Iron/Peptide Complexes

The facile losses of small molecules such as H_2O and H_2S occur upon CA of iron/peptide complexes. As was reported by Hu et al. [17, 19], facile losses of side-chain groups from metal–ion, complexes of peptides may result from direct metal–ion, side-chain interactions. Because the Cys thiol group is a reasonable base (proton affinities of the cysteine residue and propanethiol are 214 [33] and 190 [34] kcal/mol, respectively), it can accept a proton from the peptide backbone and be released as H_2S. Deuterium-labeling experiments show that the loss from a metal/peptide complex in which all active hydrogens are exchanged for D is 35 u, showing loss of HDS. One hydrogen of the expelled H_2S originates from a nonexchangable site and may involve the transfer of an α-hydrogen from the peptide chain through a four-membered ring intermediate (Scheme **1**).



Scheme 1



Scheme 2

To address mechanisms for water loss other than that described previously [16], we carried out isotope labeling experiments on the Fe^{2+}/complex of GCCAL. Deuterium exchange of the active hydrogens of the peptide shows that the [peptide-d_9 − D + Fe]$^+$ of m/z 528, decomposes to give product ions of m/z 508 and 509, indicating that there are two independent mechanisms for the loss of H_2O (Figure 5). A mechanism for loss of HDO (formation of m/z 509 ion) was described previously [16], and it involves the C-terminal OH group and the nonexchangable α-hydrogen. The loss of D_2O shows the existence of a mechanism that depends on the dramatic increases in the acidities of the amide and thiol groups caused by binding to Fe^{2+} [35]. In solution, the acidity of a ligand amide group increases (pK_a decreases to 4.0) [35] owing to their participation in multidentate chelation to Fe^{2+}. Crystal structures reveal that complexation by the metal ion causes the bond length of the peptide (C–N) bond to decrease and to take on more double-bond character, whereas the C–O bond lengthens and has less double-bond character [35]. The peptide nitrogens that are bound to Fe^{2+} do not bear a proton according to their crystal structures [35]. Rather, when peptide nitrogens are coordinated to metal ions, protons are bound at the peptide oxygens. This leads to the formation of enamides, tautomers of the –CO–NH– bond, which in the gas phase, contribute a proton to the departing water molecule. To gather additional evidence, we submitted the iron/peptide complex of GCCAL–NH_2 to CA; the loss of water remains a dominant fragmentation pathway, even in the absence of a free carboxylic acid C-terminus.

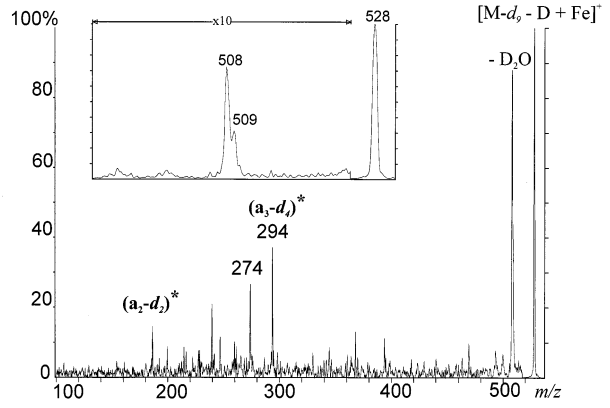


Figure 5. The CAD mass spectrum of [M-d_9 − D + Fe]$^+$ complex of GCCAL (m/z 528).

Origin of the [a_n^* − H_2O] Ions

A peak corresponding to the abundant ion [a_3^* − H_2O] of m/z 272 is in all the spectra of peptides with two adjacent Cys residues located in positions 2 and 3 from the N-terminus. The process giving rise to this ion appears to be general because Fe^{2+}/peptide complexes that contain two Cys residues at positions 3 and 4, decompose to give [a_4^* − H_2O] ions, and those with two Cys residues at positions 4 and 5 produce [a_5^* − H_2O] ions. Furthermore, both [a_3^* − H_2O] and [a_4^* − H_2O] ions were formed when the Fe^{2+}/GCCCAV complex was submitted to CA. The abundance of the [a_5^* − H_2O] ion is much lower than that of the [a_3^* − H_2O], probably owing to the participation of the N-terminal NH_2 in ion formation.

To investigate the origin of the m/z 272 ion, we carried out higher order (MS^3) collisional activation and isotope-labeling experiments. CAD of the product ion of m/z 502, which is [M − H + Fe − H_2O]$^+$ of GCCAL, gives rise to the fragment ions of m/z 484, [M − H + Fe − $2H_2O$]$^+$, m/z 361, a_4^* − H_2O, and m/z 272, a_3^* − H_2O. Subsequent activation of the m/z 290 ion (in an MS^3 experiment), which is a_3^* and comes from the original complex, leads to the formation of the low abundance ion a_2^* of m/z 187 and [a_3 − 2H + Fe − H_2O]$^+$ of m/z 272. The ion of m/z 272 is formed from that of m/z 502, [M − H + Fe − H_2O]$^+$ by sequential reaction at the site of the Cys that is involved in binding to Fe^{2+} and from the ion a_3^* undergoing H_2O loss (Scheme **2**).

Low-Energy CAD of ESI-Produced [M − H + Fe]$^+$ Ions

[M − H + Fe]$^+$ ions can be formed by electrospray ionization (ESI) as well as by FAB, and their abundance is a major fraction of that of the [M + H]$^+$. We studied four peptides GCCAL, AVAGCL, GCACLV, and GCACVCL and found that none gave the characteristic a_n^* ions that indicate the location of Cys. The most facile fragmentation instead is loss of H_2O for all peptides, and, for those containing two or more Cys, a second loss of water. The only sequence ions are $(b_{n-1})^*$ and $[(b_{n-1})^* - H_2O]$, where n is the number of amino acids in the peptide. Clearly, different fragmentation chan-

nels are available for $[M - H + Fe]^+$ ions that are activated by many low-energy than by a few high-energy collisions. This difference may be because of electronic excitation of the metal center upon high-energy CA. Because our interest in this research is the production of a* ions, we did not pursue low-energy CA any further.

Conclusion

Specific and strong interactions of Fe^{2+} with the thiol group of Cys-containing peptides and with amide and amine nitrogens occur in the gas phase. Owing to these strong interactions, Fe^{2+}-containing complexes principally fragment upon high-energy CA to give a_n^* ions that form at the sites of the cysteine residue. The product ions are stabilized and resistant to further fragmentation because the Fe–S interaction is preserved in the fragmentation. Deprotonation of the amide and thiol hydrogens occurs upon chelation to the Fe^{2+} center, explaining the extensive water loss involving the protonated carbonyl oxygen of the peptide bond in addition to the more expected expulsion of water from the unbound C-terminal carboxylic group.

The presence of more than one Cys residue in the peptide chain causes additional $[a_n^* - H_2O]$ ions to form upon CA of Fe^{2+}/peptide complexes. The effect depends on the location of the cysteines with respect to each other and, to a smaller extent, on the length of the peptide chain. Peptides that contain adjacent Cys (GCCAL, GCCACV, GCCCAV, and GCCCCA) form complexes that decompose to give a series of metal-containing a ions, depending on the position of Cys.

Future studies will consider larger peptides, peptides with Cys at the N-terminus, and peptides with other functional groups in addition to SH.

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